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<b>(54) Title:</b> METHODS OF DIAGNOSING ULCERATIVE COLITIS AND CROHN'S DISEASE  <b>(57) Abstract</b>  A novel association between inflammatory bowel disease (IBD) and a polymorphism at amino acid residue 241 or intracellular adhesion molecule-1 ICAM-1 has been discovered. In accordance with the present invention there is provided methods of screening for IBD, methods for treating IBD, antibodies specifically reactive with ICAM-1 encoded by R241 allele and kits which exploit the inventive methods.		

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METHODS OF DIAGNOSING  
ULCERATIVE COLITIS AND CROHN'S DISEASE

I. ACKNOWLEDGEMENT

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5 II. FIELD OF THE INVENTION

The present invention relates to cellular adhesion molecules. More specifically, this invention relates to a genetic polymorphism in intercellular adhesion molecule-1 ("ICAM-1") which has been found to be  
10 associated with ulcerative colitis and Crohn's disease.

III. BACKGROUND OF THE INVENTION

A. Inflammatory Bowel Disease

Inflammatory Bowel Disease ("IBD") is the collective term used to describe two chronic, idiopathic  
15 inflammatory diseases of the gastrointestinal tract: ulcerative colitis ("UC") and Crohn's disease ("CD"). UC and CD are considered together because of their overlapping clinical, etiologic, and pathogenetic features. From a therapeutic and prognostic standpoint,  
20 however, it is important to distinguish them.

IBD occurs world-wide and is reported to afflict as many as two million people. Onset has been documented at all ages; however, IBD predominately begins in young adulthood. The three most common presenting symptoms of  
25 IBD are diarrhea, abdominal pain, and fever. The diarrhea may range from mild to severe and is often accompanied by urgency and frequency. In UC, the diarrhea is usually bloody and may contain mucus and

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purulent matter as well. Anemia and weight loss are additional common signs of IBD. Reports of an increasing occurrence of psychological problems, including anxiety and depression, are perhaps not surprising secondary effects of what is often a debilitating disease that occurs in people in the prime of life.

## B. Methods of Diagnosing IBD

A battery of laboratory, radiological, and endoscopic evaluations are combined to derive a diagnosis of IBD and to assess the extent and severity of the disease. Nevertheless, differentiating UC from CD, as well as other types of inflammatory conditions of the intestines, such as irritable bowel syndrome, infectious diarrhea, rectal bleeding, radiation colitis, and the like, is difficult, because the mucosa of the small and large intestines reacts in a similar way to a large number of different insults. Once other types of bowel disorders have been ruled out, the final diagnosis is often made on the basis of the progression of the disease. In many patients, though, the colitis must still be regarded as indeterminate because of the overlapping features of UC and CD, particularly with CD of the colon.

### 1. Early Symptoms of IBD

The leading early symptoms of UC and CD are chronic recurrent diarrhea, bloody diarrhea, recurrent abdominal pain, nausea, weight loss, general evidence of inflammation without any obvious explanation (fever, raised ESR, leucocytosis, thrombocytosis and dysproteinemia or anemia). Among these symptoms, diarrhea and anemia are more characteristic of UC while pain and weight loss and marked evidence of inflammation are more common in CD. While the history and physical examination of a patient can help, the final

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confirmation of the diagnosis has traditionally been made endoscopically, histologically and, in relation to the small intestine, radiologically as well.

## 2. Endoscopic and/or Radiologic Examination

5       An endoscopic examination of the bowel can reveal important changes in mucosal appearances which can aid the physician in diagnosing IBD.

10       Unlike CD, UC is a disease of the mucosa and is confined to the large intestine. UC usually begins in the rectum, although it may involve the entire colon at the time of presentation. When UC spreads, it spreads proximally and continuously, without skipping areas. Hence, it is important to take multiple biopsy specimens from different sites of involved and apparent uninvolved  
15       mucosa. In some patients, UC remains localized to the rectum or to the left side of the colon.

20       The mucosa in acutely active UC appears to be hyperemic, granular and friable, while CD shows lymphoid follicles, aphthoid lesions and flat ulcers. Despite its name, inflammation rather than ulceration is the cardinal feature of UC. Ulcerations may or may not be present in UC. Occasionally, inflammation and ulceration vary in severity in different parts of the colon, including the rectum, giving the false impression  
25       of skip areas and rectal sparing, the latter of which are features of CD and are of diagnostic importance for that disorder.

30       The mucosa of CD exhibits patchy involvement with edema, hyperemia, and ulcerations. The ulceration is a prominent feature of CD. Both superficial and deep undermining or cleft-like ulcers occur. They may be linear or serpiginous. Occasionally, the combination of edema with ulcerations creates a cobblestone appearance that is seen radiologically and

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endoscopically. Inflammatory polyps, as in UC, may occur.

### 3. Histological Examination

The cardinal histological features in UC include  
5 vascular congestion, edema, goblet cell mucin depletion,  
crypt abscess formation, and inflammatory cell  
infiltration of the lamina propria. Crypt abscesses are  
collections of neutrophils that invade the crypt  
10 epithelium and accumulate within the lumen of the  
crypts. Ulcerations, if they occur, are superficial and  
only become penetrating to the propria muscularis when  
the disease is fulminant and acute toxic dilatation of  
the colon occurs.

Histology in CD shows the characteristic findings  
15 of granuloma formation with epithelioid and giant cells.  
However, these features are found in only 20-40% of  
biopsies. Transmural inflammation is also typical of  
CD, and even more typical is its disproportionate  
distribution (submucosa > mucosa). The mucosa shows  
20 infiltration by granulocytes with preservation of normal  
numbers of goblet cells. Lymphocytes and plasma cells  
are found in the lamina propria, and lymphoid aggregates  
are present. Lastly, aphthoid lesions are a typical  
histological feature in the early stages.

### 25 4. Determination of ANCA Status

The presence of anti-neutrophil cytoplasmic  
antibodies ("ANCAs") can easily be detected in a blood  
sample, for example, by immunofluorescence assay or a  
fixed neutrophil ELISA as detailed in Saxon, et al., J.  
30 Allergy Clin. Immunol. Vol. 86 No. 2, pp. 202-210 (1990)  
and incorporated herein by reference. The prevalence of  
positive ANCA in patients with UC ranges from 50 to 86%.  
This UC-associated ANCA has perinuclear immunofluores-  
cence binding pattern which is different from other

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ANCAs. Moreover, the presence of ANCA is highly specific for UC compared with other forms of colitis. Although a proportion of CD exhibit ANCA, it is at a much lower titre than UC.

5           Thus the ANCA status of a patient (positive indicating UC and negative indicating CD) is another factor that aids the physician in the diagnosis of IBD. ANCAs also have an increased frequency among the clinically healthy relatives of UC patients compared with  
10           environmental and ethnically matched controls. Therefore, ANCA status, in combination with family history of IBD, has also aided physicians in predicting a subjects' susceptibility to IBD.

          To date, a diagnosis of UC or CD is quite  
15           subjective and depends upon a host of procedures aimed at confirming the suspected diagnosis. The initial symptoms are often confused for non-chronic bowel disorders by physicians unfamiliar with IBD. Consequently, IBD often goes mistreated and undiagnosed  
20           until the disease shows its chronicity which results in referral of the patient to a specialist. The imprecise and subjective nature of endoscopic and radiologic examination can result in a misdiagnosis between UC and CD or indeterminate diagnosis even when the IBD is  
25           suspected.

          Histological examination and ANCA status do provide greater certainty of an accurate diagnosis, but the problems of differentiating between the two diseases based on the histological findings are often under-  
30           estimated. There is no single histological criterion which is proof of one or the other disease. The epithelial cell granuloma for example, which is often accorded a key role in the diagnosis of CD is only to be found in about 20% of bioptic specimens from such  
35           patients. They can also occur in other diseases. Unfortunately, the patient must often suffer as the

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disease progresses before a definitive diagnosis can be made.

5 The selective identification of UC as opposed to CD or other inflammatory conditions of the intestines carries important prognostic and therapeutic implications. For example, when colectomy is indicated, the type of IBD involved determines which surgical options are appropriate. Surgery (total colectomy) does represent a cure in UC, though a dramatic one. In CD, 10 surgery is never curative. Continent procedures such as the ileorectal pull-through (mucosal proctectomy) or the Kock pouch may be desirable in UC, but are contra-indicated in CD.

15 C. Inflammation and Intercellular Adhesion Molecules

Although the cause(s) of UC and CD is not known, there is general agreement that genetics is important in a person's susceptibility to UC and CD and that the immune system is responsible for mediating the tissue 20 damage in these diseases. A wide range of immunologic abnormalities have been reported in these disorders, but none has yet been sufficiently reliable to be of diagnostic value.

25 What characterizes the various forms of IBD is a failure to down regulate the normal self-limited inflammatory response of the gut. Inflammation is the response of vascularized tissue to infection or injury. Clinically it is accompanied by four classic signs: redness, heat, pain and swelling. Its course may be 30 acute or chronic.

At the cellular level, inflammation involves the adhesion of leukocytes (white blood cells) to the endothelial wall of blood vessels and their infiltration into the surrounding tissues. In normal inflammation,



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the infiltrating leukocytes phagocytize invading organisms or dead cells, and play a role in tissue repair and the immune response. However, in pathologic inflammation, infiltrating leukocytes can cause serious and sometimes deadly damage.

Recognizing that leukocyte infiltration is the cause of much inflammation-related pathology and that leukocyte adhesion is the first step in infiltration, investigators have recently focused attention on the mechanism of leukocyte binding to the endothelium cell surface. Studies show that binding is mediated by cell-surface molecules on both endothelial cell and leukocytes, which act as receptor and ligand. For a review of the roles of leukocyte integrins, immunoglobulin (Ig) gene family members, and selections in the leukocyte rolling, attachment, and transendothelial migration, the reader is directed to Springer, T.A., et al., *Nature*, 346: 425-433 (1990); Springer, T.A., et al., "Leukocyte Adhesion Molecules Structure Function and Regulation," New York: Springer-Verlag (1990); Lasky, L.A., *Science* 258: 964-969 (1992); McEver, R.P., *Curr. Opin. Cell. Biol.* 4: 840-849 (1992); Lipsky, P.E., "Structure, Function and Regulation of Molecules Involved in Leukocyte Adhesion," New York: Springer-Verlag (1993); Hogg, N. and Landis, R.C., *Curr. Opin. Immunol.* 5: 383-390 (1993).

Transendothelial migration begins with leukocyte rolling which is largely brought about by the selectins Lasky, et al., 1992. The later stages of endothelial migration involve activation of leukocyte integrins and binding of leukocytes to the endothelial cells which express Ig-like proteins including intercellular adhesion molecule-1 ("ICAM-1"). The interaction of leukocyte integrins with the Ig-like proteins leads to firm attachment and actual migration across the endothelial surface (see, Smith, C.W., et al., *J. Clin. Invest.* 82: 1746-1756 (1988) and Smith, C.W.,

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"Transendothelial Migration." ed. Harlan, J.M. and Liu, D.Y. New York: W.H. Freeman & Co. pp. 83-115 (1992). Thus, a genetic variation in these inflammatory cell adhesion molecules would be an important variable in the susceptibility, diagnosis and treatment of multifactorial disease processes like IBD which involve inflammatory or immunological responses.

The availability of a diagnostic marker that would readily distinguish UC from CD of the colon, independent of or in combination with existing diagnostic methods, would represent a major clinical advance which would aid in therapeutic management of IBD and the design of more specific treatment modalities. Accordingly, there has existed a need for a convenient and reliable method to distinguish UC from CD for diagnostic, prognostic and therapeutic purposes.

#### IV. BRIEF DESCRIPTION OF THE INVENTION

A novel association between Inflammatory Bowel Disease ("IBD") and a polymorphism in intercellular adhesion molecule-1 ("ICAM-1") has been discovered. In accordance with the present invention there are provided methods of screening for IBD, comprising assaying nucleic acid of a subject for the presence or absence of R241 allele of the ICAM-1 gene, wherein the presence of said R241 allele is indicative of IBD.

Also provided are novel antibodies specifically reactive with ICAM-1 encoded by R241 allele of the ICAM-1 gene. In addition, methods of screening for IBD, comprising assaying a tissue sample from a subject for the presence or absence of arginine at amino acid residue 241 of ICAM-1 wherein the presence of arginine at amino acid residue 241 of ICAM-1 is indicative of IBD.

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Therapeutic methods for treating IBD and kits for exploiting the inventive methods of the present invention are also provided.

#### V. DETAILED DESCRIPTION OF THE INVENTION

5           An association between IBD and a polymorphism in ICAM-1 has been discovered. This polymorphism provides the basis for convenient and reliable methods of screening for IBD, providing physicians with valuable information in the diagnosis of IBD and the  
10           determination of susceptibility to IBD. This polymorphism can also be exploited in therapeutic treatments of IBD.

          Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is  
15           commonly understood by one of skill in the art to which this invention belongs.

          The amino acids, which occur in various amino acid sequences appearing herein, are identified according to their well-known, three letter or one letter  
20           abbreviations. The nucleotides, which occur in the various nucleic acid sequences described herein, are designated with the standard single-letter designations used routinely in the art.

          The gene structure for human ICAM-1 was  
25           characterized in Voraberger, et. al., J. Immunol. 147: 2777-86 (1991) and the gene was localized to chromosome 19 as reported in Greve, J.M., et al., Cell 56: 839-847 (1989). The ICAM-1 gene was found to be distributed over seven exons separated by six introns. The five  
30           extracellular Ig-like domains of ICAM-1 gene are each encoded by its own exon. A representative genomic DNA sequence of exon 3, intron 3, exon 4, intron 4 and exon 5 (in consecutive order reading 5' to 3') for human ICAM-1 is set forth in SEQ ID NO 1. The complete

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nucleotide sequence of the ICAM-1 gene is published in EMBL GenBank database under accession numbers X59286, X59287 and X59288.

5 The polymorphism of ICAM-1 found to be associated with IBD is located in exon 4 of the human ICAM-1 gene, wherein the codon corresponding to amino acid 241 of ICAM-1 may encode Gly (e.g., GGG, GGA, GGC, GGT) or Arg (e.g., AGG, AGA, CGT, CGC, etc.). Thus, the polymorphism is referred to herein as "G/R 241." A  
10 nucleic acid encoding a glycine at amino acid residue 241 of ICAM-1 is referred to herein as the "G241 allele" or the "G241 allele of the ICAM-1 gene." A nucleic acid encoding an arginine at amino acid residue 241 of ICAM-1 is referred to herein as the "R241 allele" or the "R241  
15 allele of the ICAM-1 gene." The term "codon 241" as used herein refers to a codon in a nucleic acid sequence which encodes amino acid residue 241 of ICAM-1.

This G/R 241 polymorphism is of particular significance because it occurs in Ig domain 3 which is  
20 demonstrated to be of importance in binding to the Mac-11 form of leukocyte integrin. (See, Diamond, M.S., et al., Cell 65: 961-971, (1991).) As indicated below, the common allele is G241. Glycine is present at the analogous position in ICAM-1 from chimpanzee (Hammond  
25 L., McClelland A., unpublished. GenBank Accession No. 86848), dog (Smith, C.W., et al., J. Clin. Invest. 88: 1216-1223 (1991)), mouse (Ballantyne, C.M., et al., Genomics 9: 547-550 (1991)), and rat (Kita, Y., et al., Biochim. Biophys. Acta 1131: 108-111 (1992)), and in  
30 human ICAM-3 (Fawcett, J., et al., Nature 360: 481-84 (1992)) which indicates that the nonconservative substitution of Arg at amino acid residue 241 affects the adhesive function ICAM-1.

A detailed description of the experimental methods  
35 used to discover the association of the G/R 241 polymorphism with IBD is provided in the "Examples" set

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forth below. A total of 118 UC patients and 127 CD patients from the clinical IBD programs at Cedars-Sinai Medical Center and the University of California, Los Angeles were involved. The study protocols were  
5 approved by each institution's Human Subject Review Committee. The diagnosis of UC was documented by conventional endoscopic, histological, and clinical criteria. The UC patients studied did not have multiple  
10 sclerosis, systemic lupus erythematosus, or other recognized autoimmune diseases.

To select an ethnically, socioeconomically matched control group for the association study, 71 controls were ascertained from the spouses or acquaintances of the patients. An individual was used as control only if  
15 he/she did not have inflammatory bowel disease, multiple sclerosis, systemic lupus erythematosus, or other recognized autoimmune diseases. The distribution of age, gender, and ethnicity (Jewish/non-Jewish) were comparable between patients and controls. All patients  
20 and controls analyzed are Caucasians.

Briefly stated, genomic DNA from test subjects and control subjects was isolated and a region encoding amino acid 241 of ICAM-1 was amplified. Each subject's DNA was tested using allele-specific oligonucleotide  
25 probes to determine which of the two allele (G241 or R241) was encoded. The ANCA status (positive or negative) of each subject was determined using fixed-neutrophil ELISA. Positive ANCA status was confirmed by indirect immunofluorescence.

30 It was determined that the R241 allele is the less frequent allele at codon 241 as compared with the G241 allele. Table 1 shows the homozygous and heterozygous allele occurrence and the allele frequency for UC, CD and control subjects.

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Table 1. Comparison between UC or CD and Controls

		<u>Genotype (%)</u>				<u>Allele (%)</u>	
<u>G/R241</u>		<u>N</u>	<u>GG</u>	<u>GR</u>	<u>RR</u>	<u>G</u>	<u>R</u>
	UC	117	85.5	12.8	1.7	92.0	8.0+
5	CD	127	81.1	17.3	1.6	89.8	10.2+
	Control	70	87.1	12.9	0.0	93.6	6.4

+comparisons with control group are not statistically significant

10 The R241 allele frequency was 8.0% in patients with UC (considered as one group), 10.2% in patients with CD (taken as one group), and 6.4% in the ethnically matched control group. The difference between any two of the groups is not statistically significant.

15 However, when UC and CD patients were divided into subgroups depending on the presence or absence of ANCA, a significant difference in the frequencies of the codon 241 polymorphism was observed between ANCA(+) UC and ANCA(-) UC, and between ANCA(+) CD and ANCA(-) CD.

20 Table 2. Comparisons within UC and within CD as a function of ANCA Status

		<u>Genotype (%)</u>				<u>Allele (%)</u>	
		<u>N</u>	<u>GG</u>	<u>GR</u>	<u>RR</u>	<u>G</u>	<u>R</u>
	ANCA(+) UC	89	89.9	9.0	1.1	94.4	5.6
	ANCA(-) UC	25	72.0	24.0	4.0	84.0	16.0
25							P=0.016
	ANCA(+) CD	27	63.0	33.3	3.7	79.6	20.4
	ANCA(-) CD	90	84.4	14.4	1.1	91.7	8.3
							P=0.014

30 As shown in Table 2, ANCA(-) UC exhibited a statistically significant increase of R241 allele

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compared to the ANCA(+) UC (16.0% vs. 5.6%,  $p=0.016$ ). ANCA(+) CD had a similar statistically significant increase compared to ANCA(-) CD (20.4% vs. 8.3%,  $p=0.014$ ). The remaining groups, i.e., ANCA(+) UC and ANCA(-) CD, have a similar frequency of the polymorphism to controls (5.6% in ANCA(+) UC, 8.3% in ANCA(-) CD, and 6.4% in controls). The significance of any associations of ICAM-1 alleles with UC or CD each taken as one group, or with subsets of UC or CD stratified by ANCAs, was tested using a chi-square test or Fisher's exact probability test when appropriate.

The genotype frequencies described in Table 2 follow Hardy-Wenberg distributions in all subgroups. When the comparison between ANCA(+) and ANCA(-) groups was made by examining the frequency of patients with or without allele R241, the differences within UC and within CD, as set forth in Table 3, were again significant and odd ratios were 3.5 for ANCA(-) UC and 3.2 for ANCA(+) CD. The Mantel-Haenszel test was performed for stratified analyses.

Table 3. Comparisons within UC and within CD stratified on ANCA status (presence or absence of an allele)

		<u>R241 allele at codon 241</u>		P value	Odd Ratio (95%CI)
		presence N (%)	absence (%)		
ANCA(+)	UC	89 10.1	89.9	0.023	3.5 (1.2, 10.5)
ANCA(-)	UC	25 28.0	72.0		
ANCA(+)	CD	27 37.0	63.0	0.015	3.2 (1.2, 8.4)
ANCA(-)	CD	90 15.6	84.4		

In accordance with the present invention, there are provided methods of screening for IBD comprising assaying nucleic acid of a subject for the presence or absence of R241 allele of the ICAM-1 gene, wherein the presence of said R241 allele is indicative of IBD. Both UC and CD may be screened for at the same time, or as in alternative embodiments of the invention, UC and CD may

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be screened for separately. Nevertheless, the presence of the R241 allele is indicative of these diseases.

The term "nucleic acid" as used herein refers to DNA or RNA. Thus the methods of the present invention  
5 may be performed, for example, by using genomic DNA as well as cDNA or mRNA.

Those of skill in the art will understand that there are numerous well known methods to assay for the presence or absence of the G/R 241 polymorphism given  
10 the sequence information provided herein. Thus, while exemplary assay methods are described herein, the invention is not so limited.

In one embodiment of the invention, the presence or absence of R241 allele in a subject's nucleic acid can  
15 be detected simply by starting with any nucleated cell sample, obtained from a subject, from which genomic DNA, for example, can be isolated in sufficient quantities for analysis. The presence or absence of the R241 allele can be determined by sequence analysis of genomic  
20 DNA, accomplished via Maxam-Gilbert or another conventional technique.

In another embodiment of the present invention, assaying for the presence or absence of the R241 allele includes amplification of the subject's nucleic acid,  
25 wherein the subject's nucleic acid comprises a sequence that encodes at least nucleotide 778 of ICAM-1 cDNA. Nucleotide 778 of ICAM-1 cDNA encodes (corresponds) to the nucleotide in the first position of codon 241.

The term "encode" in its various grammatical forms  
30 as used herein includes nucleotides and/or amino acids which correspond to other nucleotides or amino acids in the transcriptional and/or translational sense, despite the fact that they may not strictly encode for one another. For example, a genomic DNA nucleotide would be



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considered to "encode" a nucleotide of cDNA (e.g., nucleotide 635 of SEQ ID NO 1 "encodes" or corresponds to nucleotide 778 of SEQ ID NO 2) despite the indirect nature (through mRNA) of that code.

5        Using the sequence data given in SEQ ID NO 1-5, one can amplify nucleic acid encoding at least nucleotide 778 of ICAM-1 cDNA. The amplified nucleic acid can then be assayed by any of a variety of methods to ascertain the genotype, including for example, allele-specific  
10        oligonucleotide probing (ASO), differential restriction endonuclease digestion (DRED), and ligase-mediated gene detection (LMGD). Additional methods of analysis would also be useful in this context, such as fluorescence resonance energy transfer (FRET) as disclosed by Wolf  
15        et. al., Proc. Natl. Acad. Sci. USA 85: 8790-94 (1988).

Amplification of nucleic acid may be achieved using conventional methods, see, e.g., Maniatis, et al., Molecular Cloning: A Laboratory Manual 187-210 (Cold Spring Harbour Laboratory, 1982). For example, mRNA  
20        from epithelial cells can be converted to cDNA and then enzymatically amplified to produce microgram quantities of cDNA encoding ICAM-1.

Amplification is preferably accomplished via the polymerase chain reaction ("PCR") method disclosed by  
25        U.S. Pat. Nos. 4,698,195 and 4,800,159, the respective contents of which are hereby incorporated herein by reference. More specifically, oligonucleotide primer pairs can be constructed that allow enzymatic amplification of a subject's nucleic acid that encodes  
30        at least nucleotide 778 of ICAM-1 cDNA. The amplified nucleic acid can then be assayed to determine the presence or absence of the R241 allele.

Primer pairs suitable for use in the practice of the present invention are linear oligonucleotides  
35        ranging in length from about 15 to about 25 nucleotides

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in length. One of the primers in the pair should be complementary to a nucleotide sequence upstream of the nucleic acid that encodes at least nucleotide 778 of ICAM-1 cDNA. And the other should be complementary to a sequence located down stream of this target site.

The sequences complementary to the primer pairs may be separated by as many nucleotides as the PCR technique will allow. However, one of skill in the art will understand that there are practical limitations of subsequent assaying procedures which may dictate the number of nucleotides between the sequences complementary to the primer pairs. It is presently preferred that the primers are equidistant from the nucleotide(s) targeted for amplification.

A pair of primers suitable for use in the practice of the present invention is set forth in SEQ ID NOS 4 and 5. These primers may be used as a pair or each in combination with another suitable primer. SEQ ID NO 4 is suited for use with genomic DNA and mRNA. The sequence with which the primer of SEQ ID NO 4 anneals is in intron 3 upstream of codon 241. SEQ ID NO 5 is suitable for use with genomic DNA, cDNA or mRNA. The sequence with which the primer of SEQ ID NO 5 anneals is in exon 4 downstream of codon 241.

SEQ ID NO 1 sets forth a representative genomic DNA sequence of exon 3, intron 3, exon 4, intron 4 and exon 5 (in consecutive order reading 5' to 3') for human ICAM-1. SEQ ID NO 2 sets forth a cDNA sequence encoding ICAM-1. From this sequence information, one of skill in the art would be able to produce numerous primers suitable for use in the amplification of nucleic acid sequence encoding at least nucleotide 778 of ICAM-1 cDNA without undue experimentation.

Another embodiment of the invention utilizes allele-specific oligonucleotide ("ASO") probes for the

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R241 allele and the G241 allele to assay for the presence or absence of R241 allele of the ICAM-1 gene. Accordingly, there is provided a method of screening for IBD, comprising assaying nucleic acid of a subject for the presence or absence of R241 allele of the ICAM-1 gene by contacting the nucleic acid with an R241 allele-specific oligonucleotide probe under conditions suitable to cause the probe to hybridize with nucleic acid encoding the R241 allele of the ICAM-1 gene, but not with nucleic acid encoding the G241 allele of the ICAM-1 gene, and detecting the presence or absence of hybridization.

In yet another embodiment of the present invention, the ASO probe comprises a sequence complementary to a region of SEQ ID NO 1, wherein said region comprises at least the first nucleotide of codon 241 of SEQ ID NO 1. In still another embodiment of the present invention, the ASO probe comprises a sequence complementary to a region of SEQ ID NO 2, wherein said region comprises at least nucleotide 778 of SEQ ID NO.2

According to conventional ASO procedures, oligonucleotide probes are synthesized that will hybridize, under appropriate annealing conditions, exclusively to a particular amplified nucleic acid sequence that contains a nucleotide(s) that distinguishes one allele from other alleles. The probes are discernably labeled so that when the R241 allele-specific oligonucleotide probe hybridizes to the sequence encoding the R241 allele, it can be detected, and the specific allele is thus identified.

In another embodiment of the invention, either of the subject's amplified nucleic acid or the ASO probes can be bound onto two solid matrixes (e.g., nylon, nitrocellulose membrane and the like) by standard techniques and then each matrix can be placed into separate hybridization reactions with an ASO probe or

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amplified nucleic acid, respectively. For example, if the amplified nucleic acid were bound onto a solid matrix, one hybridization reaction would utilize an oligonucleotide probe specific for to R241 allele  
5 encoding Gly under conditions optimal for hybridization of this probe to its complement. The other hybridization reaction would utilize an oligonucleotide specific to G241 allele encoded by GGG under conditions optimal for hybridization of that probe to its  
10 complement. Accordingly, the ASO probes may bear the same label, but will still be distinguishable because they are hybridized in separate chambers.

In this manner, not only can it be determined whether the subject's nucleic acid encodes the R241  
15 allele, but it can also be determined whether the subject is a heterozygote or a homozygote. If an ASO probe is found to bind to subject's nucleic acid on only one matrix, then the subject is homozygous for that particular allele which the ASO probe was designed to  
20 bind. If the ASO probes are found to hybridize the subject's nucleic acid on both membranes, then the subject is heterozygous. An example of this technique applied to the detection of cystic fibrosis heterozygotes is Lemna, W.K., et al., N. Eng. J. Med.  
25 322: pp. 291-296 (1990).

The ASO probes of the present invention can be about 7 to about 35 nucleotides in length, preferably about 15 to 20 nucleotides in length, and are complementary to a nucleic acid sequence encoding at  
30 least nucleotide 778 of ICAM-1 cDNA. "R241 ASO probes" are specific for the R241 allele. An example of an "R241 ASO probe" is set forth in SEQ ID NO 7. "G241 ASO probes" are specific for the G241 allele. An example of an "G241 ASO probe" is set forth in SEQ ID NO 6. Those  
35 of skill in the art will understand that other ASO probes may be designed using the sequence information provided herein. For probe design, hybridization

techniques and stringency conditions, see, Ausubel, et al., (eds.) "Current Protocols in Molecular Biology" Wiley Intersciences, New York, sections 6.3 and 6.4 (1987, 1989). Additional approaches to probe design and  
5 detection can also be used, e.g., ligase-mediated gene detection (LMGD), as disclosed by Landeregren, et al., Science 241: 1077-80 (1988) and fluorescence resonance energy transfer (FRET), as disclosed by Wolf, et al., Proc. Nat. Acad. Sci. (USA) 85: 8790-94 (1988).

10       The ASO probes may be discernably "labelled." As used herein, the term "label" in its various grammatical forms refers to single atoms and molecules that are either directly or indirectly involved in the production  
15 of a detectable signal to indicate the presence of a complex (e.g. radioisotope, enzyme, chromogenic or fluorogenic substance, a chemiluminescent marker, or the like). Any label can be linked to or incorporated in an ASO probe, These atoms or molecules can be used alone or in conjunction with additional reagents. Such labels  
20 are themselves well-known in clinical diagnostic chemistry.

"Conditions suitable to cause the probe to hybridize with nucleic acid encoding the R241 allele of the ICAM-1 gene, but not with nucleic acid encoding the  
25 G241 allele of the ICAM-1 gene" is intended to mean that the hybridization reaction conditions should be set for preferential binding of the R241 allele-specific oligonucleotide probe to only the R241 allele and not the G241 allele. One of skill in the art can readily  
30 determine such conditions for hybridization based upon the nature of the probe used, factoring into consideration time, temperature, pH and the like.

In still another embodiment of the present invention, there is provided a method of screening for  
35 IBD, comprising assaying nucleic acid of a subject for the presence or absence of R241 allele of the ICAM-1

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gene. Such assays are carried out by cleaving subject's nucleic acid with a restriction endonuclease, wherein the restriction endonuclease differentially cleaves nucleic acid encoding R241 allele as compared to nucleic acid encoding G241 allele. The subject's nucleic acid employed for such assays comprises a sequence encoding at least nucleotide 778 of the ICAM-1 cDNA.

DRED analysis is accomplished in the following manner. If conditions occur including (1) a particular amplified nucleic acid contains a sequence variation that distinguishes an allele of a polymorphism and (2) this sequence variation is recognized by a restriction endonuclease, then the cleavage by the enzyme of a particular nucleic acid sequence can be used to determine the allele. In accomplishing this determination, amplified nucleic acid of a subject is digested and the resulting fragments are analyzed by size or movement through a gel. The presence or absence of nucleotide fragments, corresponding to the endonuclease cleaved fragments, determines which allele is present.

A restriction endonuclease suitable for use in the practice of the present invention can readily be identified by one of skill in the art, for example, by synthesizing two nucleic acid sequences of equal length which are identical to one another and to cDNA sequence encoding codon 241, except that one sequence encodes the R241 allele and the other encodes the G241 allele. A prospective restriction endonuclease is then contacted with each of the sequences, the sequences are examined and the restriction endonuclease deemed suitable if the two sequences are cleaved differently. The same procedure may be followed to identify restriction endonucleases suitable for use in the practice of the present invention with genomic DNA.

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The present invention also provides methods of screening for IBD, comprising assaying nucleic acid of a subject for the presence or absence of R241 allele of the ICAM-1 gene by hybridizing the nucleic acid with a pair of oligonucleotide probes to produce a construct, wherein a first probe of the pair is labeled with a first label and a second probe of the pair is labeled with a second label, such that the first label is distinguishable from said second label, and the probes hybridize adjacent to each other at a nucleotide encoding amino acid residue 241 of ICAM-1. Thereafter, the construct is reacted with a ligase in a reaction medium, and then the reaction medium is analyzed to detect the presence or absence of a ligation product comprising said first probe and said second probe.

In the course of an LMCD-type assay, as disclosed by Landegren et al., Science 241: 1077-80 (1988), a pair of oligonucleotide probes are synthesized that will hybridize adjacent to each other, for example, on a cDNA segment under appropriate annealing conditions, at the specific nucleotide that distinguishes the R241 allele from the G241 allele of ICAM-1 gene. Each of the pair of specific probes is labeled in a different manner, and when it hybridizes to the allele-distinguishing cDNA segment, both probes can be ligated together by the addition of a ligase.

When the ligated probes are isolated from the cDNA segment, both types of labeling can be observed together, confirming the presence of the R241 allele-specific nucleotide sequence. Where the above-described pair of differently labeled probes bind to a nucleic acid containing a distinguishing nucleotide of the G241 allele, the probe pair is not ligatable and, after the probes are isolated from the cDNA segments, both types of labeling are observed separately.

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An exemplary LMGD-type assay for detecting the presence or absence of R241 allele of the ICAM-1 gene in a subject's nucleic acid, in accordance with the present invention, entails the use of a pair of oligonucleotide probes that bind to a nucleic acid adjacent to each other at a nucleotide encoding nucleotide 778 of ICAM-1 cDNA, wherein one probe is radioactively <sup>32</sup>P-labeled and the other probe is biotin-labeled. The biotin labeled probe hybridizes nucleotides encoding 758-778 of ICAM-1 cDNA, wherein nucleotide 778 is an adenine, which distinguishes the R241 allele. The <sup>32</sup>P-labeled probe hybridizes nucleotides encoding nucleotides 779-799 of ICAM-1 cDNA and, therefore will hybridize adjacent to the biotin labeled probe. These probes are then added under annealing conditions such that they hybridize adjacent to each other spanning nucleotides encoding nucleotides 758-779 of ICAM-1 cDNA.

When the R241 allele sequence is present in the amplified nucleic acid, then the addition of a ligase will result in the biotin labeled probe being covalently bound to the <sup>32</sup>P-labeled probe. The ligation is possible, because the ends of the probes that are adjacent to each other (hybridized to nucleotides 778 and 779) are both hybridized to the nucleotides encoding the ICAM-1 cDNA. In the case where these two probes hybridize to the G241 allele, the biotin-labeled probe end at nucleotide 778 is not hybridized appropriately, preventing ligation from occurring.

When this pair of probes binds completely to the nucleic acid encoding R241 allele, therefore, the probes are ligated. When the probes are separated from the nucleic acid encoding R241 allele and detected, both the biotin/streptavidin and the <sup>32</sup>P-labeling are present together. When the G241 allele sequence is hybridized, on the other hand, the probes cannot be ligated, and the biotin/streptavidin-and <sup>32</sup>P-labeling are observed



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separately. In this manner, R241 allele and G241 allele can be distinguished.

5 The invention further includes antibodies which are capable of binding ICAM-1 encoded by R241 allele, but not to ICAM-1 encoded by G241 allele. Such an antibody may be easily produced by one of skill in the art by preparing a peptide, protein conjugate which is specific to the unique amino acid in ICAM-1 encoded by R241 allele and immunizing an animal as discussed below. The  
10 invention includes the hybridoma cell line which produces the antibody of the same specificity, the antibody produced by the hybridoma cell line and the method of production.

15 Antibodies raised against the R241 allele or ICAM-1 encoded by the R241 allele are expected to have utility in the diagnosis, prevention and treatment of IBD. In this context, the term "antibody" encompasses monoclonal antibodies, polyclonal antibodies and humanized antibodies. Preferably, for therapeutic applications,  
20 the antibodies employed will be humanized, monoclonal antibodies.

The above-described antibodies can be prepared employing standard techniques, as are well known to those of skill in the art, using R241 allele or ICAM-1  
25 encoded by the R241 allele ("R241 ICAM-1"), or fragments thereof, as antigens for antibody production. Antibodies of the present invention are typically produced by immunizing a mammal with an inoculum containing R241 ICAM-1, or fragments thereof and  
30 thereby inducing in the mammal antibody molecules having immunospecificity for R241 ICAM-1 or polypeptide fragment thereof.

For example, antibodies raised in rabbits against a synthetic peptide recognize the synthetic peptide and  
35 the R241 ICAM-1 on an equimolar basis, and, preferably,

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they are capable of inhibiting the activity of the native protein. Antibodies to R241 ICAM-1 may be obtained, for example, by immunizing three month old male and female white New Zealand rabbits with the synthetic peptide or fragment thereof to which Tyr has been added at the C-terminus in order to couple it, as an antigen, to BSA by a bisdiazotized benzidine (BDB) linkage by reaction for 2 hours at 4°C. The reaction mixture is dialyzed to remove low molecular weight material, and the retentate is frozen in liquid nitrogen and stored at -20°C. Animals are immunized with the equivalent of 1 mg of the peptide antigen according to the procedure of Benoit et al. P.N.A.S. USA, 79, 917-921 (1982). At four week intervals, the animals are boosted by injections of 200 µg of the antigen and bled ten to fourteen days later. After the third boost, antiserum is examined for its capacity to bind radioiodinated antigen peptide prepared by the chloramine-T method and then purified by CMC-ion exchange column chromatography. The antibody molecules are then collected from the mammal and isolated to the extent desired by well known techniques such as, for example, by using DEAE Sephadex to obtain the IgG fraction.

To enhance the specificity of the antibody, the antibodies may be purified by suitable techniques, for example, by immunoaffinity chromatography using solid phase-affixed immunizing polypeptide. The antibody is contacted with the solid phase-affixed immunizing polypeptide for a period of time sufficient for the polypeptide to immunoreact with the antibody molecules to form a solid phase-affixed immunocomplex. The bound antibodies are separated from the complex by standard techniques.

A radioimmunoassay is established with the antisera and serum from subsequent bleeds from the same rabbits. The native protein is recognized by the antibodies on an

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equimolar basis as compared to the synthetic peptide antigen.

5 In the case of monoclonal antibody production, one proceeds by isolating lymphocytes and fusing them with myeloma cells, producing hybridomas. The cloned hybridomas are then screened for production of antibodies specific for sequences encoded by the R241 allele.

10 The antibody so produced can be used, inter alia, in diagnostic methods and assay methods to detect the presence or absence of protein encoding the R241 allele. Thus methods of screening IBD are contemplated comprising assaying a tissue sample from a subject for the presence or absence of arginine at amino acid  
15 residue 241 of ICAM-1 wherein the presence of arginine at amino acid residue 241 of ICAM-1 is indicative of IBD. A "tissue sample" suitable for use in the practice of the present invention includes any cell samples, bodily fluids or fractions thereof which contain ICAM-1  
20 and, for example, preferably including epithelial cells.

One such method employs an "ELISA" format to detect the presence or absence of arginine at amino acid residue 241 of ICAM-1. "ELISA" refers to an enzyme-linked immunosorbent assay that employs an antibody or  
25 antigen immobilized on solid matrix and an enzyme-antigen or enzyme-antibody conjugate to detect and quantify the amount of an antigen present in a sample. A description of the ELISA technique is found in Chapter 22 of the 4th Edition of Basic and Clinical  
30 Immunology by D.P. Sites et al., published by Lange Medical Publications of Los Altos, CA in 1982 and in U.S. Patent Nos. 3,654,090, No. 3,850,752; and No. 4,016,043.

35 Thus, in a preferred embodiment a tissue sample from the test subject is contacted with immobilized

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lymphocyte function-associated antigen-1 ("LFA-1"), for example, and an enzyme-labelled antibody specific for R241 ICAM-1 under conditions suitable to form a complex of LFA-1, ICAM-1 and said labelled antibody. Unbound  
5 labelled antibody is separated from said complex, and the presence or absence of arginine at amino acid residue 241 of ICAM-1 is detected by measuring the presence or absence of bound, labelled antibody. This method may be modified by use of a non-enzymatic label.

10 Alternatively, a ligand, other than LFA-1, which is recognized by ICAM-1, or a non-R241 specific antibody or the like, may be immobilized on a solid matrix, for example. Such reagents are typically immobilized on a solid matrix by adsorption from an aqueous medium  
15 although other modes of affixation applicable to proteins and polypeptides well known to those skilled in the art can be used.

Useful solid matrices are also well known in the art. Such materials are water insoluble and include  
20 cross-linked dextran (available from Pharmacia Fine Chemicals; Piscataway, N.J.); agarose; beads of polystyrene about 1 micron to about 5 millimeters in diameter (available from Abbott Laboratories; North Chicago, IL); polyvinyl chloride; polystyrene;  
25 cross-linked polyacrylamide; nitrocellulose- or nylon-based webs such as sheets, strips or paddles; tubes, plates or the wells of a microtiter plate such as those made from polystyrene or polyvinylchloride, or the like.

30 The anti-R241 ICAM-1 antibodies can also be used for the immunoaffinity or affinity chromatography purification of ICAM-1 biological materials. In addition, an anti-R241 ICAM-1 antibody according to the present invention can be used in mammalian therapeutic  
35 methods, preferably human, as a R241 ICAM-1 agonist or to neutralize or modulate the effect of R241 ICAM-1.

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"Antibody" also encompasses fragments, like Fab and F(ab')<sub>2</sub>, of anti-R241 ICAM-1 antibodies, and conjugates of such fragments, and so-called "antigen binding proteins" (single-chain antibodies) which are based on anti-R241 ICAM-1 antibodies, in accordance, for example, with U.S. Pat. No. 4,704,692.

Antibodies against ICAM-1 encoded by the R241 allele can also be employed in the generation, via conventional methodology, of anti-idiotypic antibodies (antibodies that bind an anti-ICAM-1 allele antibody), e.g., by the use of hybridomas as described above. See, for example, U.S. Patent No. 4,699,880. Such anti-idiotypic antibodies could be used to sequester anti-R241 ICAM-1 antibodies in an individual, thereby treating or preventing pathological conditions which may be associated with an immune response whereby R241 allele is recognized as "foreign" by the immune system of the individual.

The present invention further includes methods of inhibiting leukocyte function-associated antigen-1 ("LFA-1") and R241 ICAM-1 interaction, comprising the step of contacting R241 ICAM-1 containing cells with antibody specific for R241 ICAM-1.

The present invention also provides methods for treating IBD comprising administering a therapeutically effective dose of an antibody which recognizes and binds R241 ICAM-1 wherein the R241 ICAM-1 function is modified. Antibodies suitable for use in these therapeutic methods need not be raised specifically against R241 allele and may include, for example, the murine anti-ICAM-I mAb (BIRR1) currently being tested by A. Kavanaugh, L. Nichols, L. Davis, R. Rothiem and P. Lipsky of the Univ. of Texas, Southwestern Medical Center, Dallas, Texas. Alternatively or in conjunction with treatment methods described above, IBD may be treated in accordance with the present invention by

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administering a therapeutically effective dose of antisense oligonucleotide complementary to nucleic acid encoding R241 allele of the ICAM-1 gene.

Antisense oligonucleotides can be prepared as polynucleotides complementary to (a) nucleotide sequences comprising DNA which encodes R241 allele or (b) nucleotide sequences comprising R241 allele messenger RNA (mRNA). For both types, the length of an antisense oligonucleotide of the present invention is not critical so long as there is no promoter sequence (for DNA) or Shine-Delgarno site (for RNA) present. Type (a) antisense oligonucleotides would be synthesized de novo, for example, based on knowledge concerning the nucleotide sequence of the genomic DNA shown in SEQ ID NO 1. Type (b) antisense oligonucleotides could also be produced de novo (DNA or RNA), or by transforming an appropriate host organism with DNA that is transcribed into RNA which binds an ICAM-1 allele mRNA. Both type (a) and type (b) oligonucleotides within the present invention are useful as agents for "down-regulating" (turning off) the expression of ICAM-1 on the cell surface, or inhibiting either transcription [type (a)] or translation [type (b)].

The present invention contemplates therapeutic compositions useful for practicing the therapeutic methods described herein. Therapeutic compositions of the present invention may contain a pharmaceutically acceptable carrier together with a ICAM-1 antisense oligonucleotide or anti-ICAM-1 antibody, as described herein, dissolved or dispersed therein as an active ingredient. In a preferred embodiment, the therapeutic composition is not immunogenic when administered to a mammal or human patient for therapeutic purposes. To some extent this may be accomplished, for example, by commonly known techniques of "humanizing" antibodies wherein the constant regions of a antibody derived from

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an animal are replaced with constant regions from a human.

As used herein, the term "pharmaceutically acceptable" and grammatical variations thereof, as they  
5 refer to compositions, carriers, diluents and reagents, are used to represent that the materials are capable of administration to a mammal without the production of undesirable physiological effects such as nausea, dizziness, gastric upset, and the like.

10 The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well known in the art. Typically such compositions are prepared as injectables either as liquid solutions or suspensions; however, solid forms  
15 suitable for solution, or suspension, in liquid prior to use can also be prepared. The preparation can also be emulsified.

The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible  
20 with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the composition can  
25 contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and the like, which enhance the effectiveness of the active ingredient.

The therapeutic composition of the present  
30 invention can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable nontoxic salts include the acid addition salts (formed with the free amino groups of the polypeptide) that are formed with inorganic acids (such as, for example,  
35 hydrochloric acid, hydrobromic acid, perchloric acid,

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nitric acid, thiocyanic acid, sulfuric acid, phosphoric acid); organic acids (such as, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalene sulfonic acid, sulfanilic acid), and the like.

Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium hydroxide, ammonium hydroxide, potassium hydroxide, and the like; and organic bases such as mono-, di-, and tri-alkyl and -aryl amines (e.g., triethylamine, diisopropyl amine, methyl amine, dimethyl amine, and the like) and optionally substituted ethanolamines (e.g., ethanolamine, diethanolamine, and the like).

Pharmaceutically acceptable carriers are well known in the art. Exemplary of liquid carriers are sterile aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, polyethylene glycol and other solutes.

Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, and water-oil emulsions.

A therapeutically effective amount is a predetermined amount calculated to achieve the desired effect, i.e., down regulation of the inflammatory response in the gut which is mediated by ICAM-1. The required dosage will vary with the particular treatment,



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with the duration of desired treatment, age of patient, body size of patient and the like.

Those of skill in the art will appreciate that any of the foregoing inventive methods may be used not only to screen for IBD, but also to predict a subject's susceptibility to IBD. There is general agreement that genetics are important in a person's susceptibility to UC and CD. Evidence supporting this conclusion includes consistent ethnic differences which cross different geographic areas, dramatic familial aggregation, existence of genetic syndromes that feature IBD, higher monozygotic than dizygotic twin concordance rates, lack of increased frequency in spouses, affected relatives separated in space and time, and associations between IBD and genetic markers. Thus any of the inventive methods for screening for IBD may also be used as an initial screening tool to predict a subject's susceptibility to IBD.

These methods for determining susceptibility to IBD are particularly useful in combination with a subject's family history of IBD. For example, a parent suffering from IBD and who has a family history of IBD, may well have a child who is susceptible to IBD, but who has not yet shown symptoms. To alleviate the concern of the parent, as well as the child, and to take any preventative measures which might delay or prevent onset, one of the many inventive methods provided herein can be used to determine whether the child is a carrier of the R241 allele. If so, further testing can be performed, for example, ANCA status can be determined to provide further support for the predicted susceptibility.

Similarly, the screening methods provided herein are preferably used in combination with existing methods for diagnosing IBD (e.g. endoscopic, histologic, radiologic, and in particular determination of ANCA

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status) to maximize confidence in the ultimate diagnosis regarding IBD.

Kits for use in (1) screening for IBD, (2) screening for susceptibility to IBD, and (3) treating IBD are also provided by the present invention. Such kits can include all or some of the reagents primers, probes, antibodies and antisense oligonucleotides described herein for determining the presence or absence of nucleic acid encoding R241 allele or for treatment of IBD. Kits of the present invention may contain, for example, restriction endonuclease, one or more labeled oligonucleotide probes that distinguish nucleic acid encoding nucleotide 778 of ICAM-1 cDNA, ligase, R241 allele-specific oligonucleotide probe, primer for amplification of nucleic acid encoding at least nucleotide 778 of ICAM-1 cDNA, means for amplifying a subject's nucleic acid encoding at least nucleotide 778 of ICAM-1 cDNA, neutrophil, alkaline phosphatase coupled goat anti-human gamma chain specific antibody, fluorescein-labelled goat anti-human gamma chain specific antibody, anti-human gamma chain specific antibody, antisense oligonucleotides, antibody specific for, or which binds R241 ICAM-I, or combinations of any of the above.

As amenable, these suggested kit components may be packaged in a manner customary for use by those of skill in the art. For example, these suggested kit components may be immobilized on a solid matrix or provided in solution or as a liquid dispersion or the like.

A presently preferred embodiment of the inventive kits for use in screening for IBD or determining susceptibility to IBD comprises R241 allele-specific oligonucleotide probe immobilized on a solid matrix, neutrophil immobilized on solid matrix and alkaline phosphatase-coupled goat anti-human gamma-chain specific antibody.

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The invention will now be described in greater detail by reference to the following non-limiting examples.

A. EXAMPLES

5           1.   Determination Of The G/R 241 point  
              Mutation For ICAM-1

10           The identification of the G/R 241 allele of ICAM-1 was performed by amplification of genomic DNA followed by direct sequencing of the PCR product using an ABI automated sequencer. Additional sequencing information was obtained from published literature (Stauton, et al., Cell 52: 925-933 (1988); Tomassini, et al., Proc. Natl. Acad. Sci. U.S.A. 86: 4907-4911 (1989) and GenBank (Burks, et al., Nucleic Acids Res. (Suppl.) 20: 2065-  
15   2069 (1992)).

20           Amplification of the genomic DNA was performed in 20  $\mu$ l reactions under the following conditions: 10 mM Tris HCl at pH 8.3, 50 mM KCl, 1.5 mM  $MgCl_2$ , 200  $\mu$ M each dNTP, 10  $\mu$ M each primer, 50 ng genomic DNA, and 0.5 units of Amplitaq polymerase (Perkin-Elmer Cetus, Norwalk, CT). PCR conditions were as follows: denaturing polynucleotide strands at 94°C for 1 min, annealing primers at 52° C for 45 sec, and primer extending at 72° C for 45 sec for 26 cycles. One primer  
25   for each reaction was end labeled with [ $-^{32}P$ ]ATP using T4 polynucleotide kinase. The reaction products were analyzed on a 6% denaturing polyacrylamide gel as describe in Weber, J.L. and May, P.E., Am. J. Hum. Genet. 44: 388-396 (1989) and incorporated by reference  
30   herein. The size of alleles as generated by PCR were determined using a dideoxy sequencing ladder produced from M13 DNA as a template.

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2. Amino Acid Polymorphisms Analysis Of ICAM-1  
a. Amplification of ICAM-1 Encoding DNA For  
Screening Methods

5 In order to examine genomic DNA for R241 allele of  
the ICAM-1 gene, the polymerase chain reaction was used  
to amplify a region of the ICAM-1 gene encoding amino  
acid 241 which is near the junction of intron 3 and exon

4. A pair of primers were constructed: 5'-  
GATTGAAGAAGCCAGCAG - 3' (SEQ ID NO 4) complements  
10 genomic DNA encoding intron 3 of the ICAM-1 gene, and  
5'- GTCGTTGCCATAGGTGAC - 3' (SEQ ID NO 5) complements  
genomic DNA encoding exon 4 of the ICAM-1 gene, SEQ ID  
NO 1.

Amplification of the genomic DNA was performed in  
15 20 µl reactions under the following conditions: 10 mM  
Tris HCl at pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200 µM each  
dNTP, 10 µM each primer, 50 ng genomic DNA, and 0.5  
units of Amplitag polymerase (Perkin-Elmer Cetus,  
Norwalk, CT). The conditions for PCR amplification were  
20 as follows: denaturing polynucleotide strands at 94°C  
for 30 sec, annealing primers at 55° C for 30 sec, and  
primer extending at 72° C for 45 sec for 40 cycles.

b. Screening with Allele Specific  
Oligonucleotides

25 Three µl of the PCR product was applied to a Hybond  
N+ membrane using a Beckman Biomek robot. The membranes  
were air dried and treated with denaturing solution (0.5  
N NaOH) for 15 min followed by renaturation in 2X SSC  
with 0.4 M Tris at pH7.5 for 10 min.

30 Allele specific oligonucleotides were  
5' - TCCCTGGACGGGCTGTTCC - 3' (SEQ ID NO 6) to detect  
the G241 allele and 5' - TCCCTGGACAGGCTGTTCC - 3' (SEQ  
ID NO 7) to detect the R241 allele. Oligonucleotides  
were end labeled with [γ-<sup>32</sup>P] ATP, and the membranes were

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prehybridized with buffer (10% polyethylene glycol, 7% SDS, 1% bovine serum albumin, 250 mM NaCl and 250 mM sodium phosphate) at 65°C and hybridized with  $2-3 \times 10^6$  cpm/10 ml of the labeled ASO and 20 fold higher concentration of the nonradioactive ASO for the alternate allele. Hybridization was at 65°C for 30 min and hybridization was continued subsequently at 37°C. The membranes were washed with 5X SSC at room temperature followed by 2X SSC at 45°C for 30 min and used for autoradiography.

### 3. Detection of ANCAS by ELISA

The development and validation of the fixed neutrophil ELISA has been described in detail by Saxon, et al., J. Allergy Clin. Immunol. 86: 202-10 (1990) which is incorporated herein by reference.

Purified neutrophils from a single normal individual served as the source of antigen for the fixed-neutrophil ELISA assay. Neutrophils were isolated as follows.

#### 20 a. Separation of Human Peripheral Blood Lymphocytes by Ficoll-Hypaque Gradient Centrifugation

A Ficoll-Hypaque solution having a specific gravity of 1.080 was made by vigorously combining 31.8 g Ficoll 400 (Pharmacia, Sweden) and 400 ml deionized H<sub>2</sub>O and then mixing in 100 ml of 50% sodium diatrizoate hypaque (UCLA Pharmacy, Los Angeles, California). The Ficoll-hypaque solution was then filter-sterilize through a 0.22 or 0.45 um bottle top filter. (The Ficoll-hypaque solution may be stored at 4°C, protected from light.)

15 ml of the Ficoll-hypaque solution were poured into a 50 ml conical centrifuge tube and 30 ml heparinized blood was carefully laid over it. The

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contents was centrifuge at 1000 x g (2000 RPM) for 20 min. The resulting interface was removed using a serologic pipet or pasteur pipet and placed into a second 50 ml conical centrifuge tube. The interface layer was then diluted with at least an equal volume of Hanks' Balanced Salt Solution (HBSS) (Irvine Scientific, Santa Ana, California) and centrifuged at 400 x g (1200 RPM) for 5 min. Removal of the interface, dilution and centrifugation was repeated twice again. Finally, the cell pellet was resuspended in RPMI 1640 (Irvine Scientific, Santa Ana, California) + 5% fetal calf serum (GIBCO, Gathersberg, Maryland).

b. Isolation of Neutrophil

Using a pipet, serum and remaining Ficoll-Hypaque were carefully removed from red blood cell pellet resulting from procedure described immediately above. 10 ml 6% dextran was added to 15 milliliters of pellet. 1X HBSS was added to reach a final volume of 50 ml. The pellet was re-suspended and the red blood cells were allowed to settle, approximately 45 minutes to one hour.

The supernatant was then retrieved and the pellet discarded. The supernatant was topped off with 1X HBSS to 50 ml and centrifuge for 5 minutes at 1800 rpm. The supernatant was decanted and pellet tapped. The remaining red blood cells were hypotonically lysed by adding 9 ml deionized water, swirling, adding 1 ml 10X HBSS and immediately diluting with 1X HBSS to 50 ml. This was centrifuged for 5 minutes at 1000 rpm. The supernatant was discarded and the pellet re-suspended in 15 ml 1X HBSS.

c. Immobilization of Neutrophil on Microtiter Plate

Microtiter wells (Immulon 2™, Dynatech Labs, Alexandria, VA) were coated with a monolayer of

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neutrophils by the addition of 100  $\mu$ l/well of HBSS containing 250,000 neutrophils (isolated as described above). After the cells had settled and spread for 30 min at room temperature, the microtiter plates were  
5 centrifuged at 1000 rpm (300 g) for 5 min, the supernatant was aspirated from the wells, and the plates were air dried. The cells were fixed with 100% methanol for 10 min, after which the plates were air dried and stored at -20° C.

10 d. Fixed Neutrophil ELISA

For use, the plates were brought to room temperature, and 150  $\mu$ l of 0.25% bovine serum albumin in phosphate buffered saline (BSA/PBS) was added to each microtiter well for 1 hr to block non-specific binding.  
15 The blocking materials was discarded, and 100  $\mu$ l of test serum diluted in BSA/PBS (or BSA/PBS alone for blank wells) was added. To standardize the assay, a positive pool of sera from 6 individuals with UC (3 with very high and 3 with intermediate levels of ANCA) was used.  
20 This was included on each microtiter plate at a dilution of 1:100. The plates were incubated for 1 hr at room temperature in a humidified box (sufficient humidity to minimize volume loss).

The wells were then washed 3 times with 0.05% Tween  
25 20 in PBS (PSB/Tween), and 100  $\mu$ l/well of a 1:750 dilution of alkaline phosphatase coupled goat anti-human gamma chain specific antibody (Tago, Burlingame, CA) in BSA/PBS was added. The wells were allowed to incubate for 1 hr at room temperature in a humidified box. This  
30 antibody was discarded, and the wells were washed 3 times with PBS/Tween and 4 times with 0.05M Tris base in 0.9M NaCl, pH 7.5.

Substrate solution (1.5 mg/ml disodium p-nitrophenol phosphate in 0.01M Tris base, 0.0025M MgCl<sub>2</sub>,  
35 pH 8.6, 100  $\mu$ l/well) was added, and color development

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was allowed to proceed until absorbance at 405 nm in the positive control wells was 0.8-1.0 optical density units greater than in blank wells. Plates were read plate at 405 nm in an EMAX Microplate Reader (Molecular Devices, Menlo Park, California).

### 3. Confirmation Of Positive ELISA Values By Indirect Immunofluorescence

Sera that exhibited levels of binding greater than 2 Standard deviations above the mean level for the normal laboratory controls at a dilution of 1/100 in the ELISA were regarded as being positive and were also examined by indirect immunofluorescence to confirm that the antibody binding was cell-specific.

Approximately 100,000 neutrophils/slide were applied to slides using a cytocentrifuge (Cytospin 3™, Shandon, Inc. Pittsburgh, Pennsylvania) at 500 rpm for 5 minutes. Neutrophils were fixed in 100% methanol at room temperature. The methanol was discarded and coded sera were tested at a dilution of 1:20 in BSA/PBS and stained with fluorescein-labelled F(ab')<sub>2</sub> anti human gamma chain specific antibody. The antibody was rinsed off with 100-250 ml phosphate buffered saline. The slides were soaked for 5 minutes in 100-250 ml phosphate buffered saline and then allowed to air dry. The fluorescence pattern was read on a fluorescence microscope at 40X.

Although the invention has been described with reference to presently preferred embodiments, it should be understood that various modifications can be made without departing from the spirit of the invention.



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Summary of Sequences

Sequence ID No. 1 is a representative genomic DNA sequence of exon 3, intron 3, exon 4, intron 4 and exon 5 (in consecutive order reading 5' to 3') for human ICAM-I encoding the G241 allele at nucleotides 635 to 637.

Sequence ID No. 2 is a cDNA sequence encoding the R241 allele of the ICAM-1 gene.

Sequence ID No. 3 is an amino acid sequence for ICAM-1 encoded by an R241 allele of the ICAM-1 gene which was derived from the cDNA sequence set forth in SEQ ID NO. 2.

Sequence ID No. 4 is a nucleic acid sequence of a primer suitable for amplification of genomic nucleic acid encoding the R241 allele of the ICAM-1 gene.

Sequence ID No. 5 is the nucleic acid sequence of a primer suitable for use in amplification of nucleic acid encoding the R241 allele of the ICAM-1 gene.

Sequence ID No. 6 is a nucleic acid sequence of an allele-specific oligonucleotide probe suitable for use in detecting the presence or absence of nucleic acid encoding a G241 allele of the ICAM-1 gene.

Sequence ID NO 7 is a nucleic acid sequence of an allele-specific oligonucleotide probe suitable for use in detecting the presence or absence of nucleic acid encoding an R241 allele of the ICAM-I gene.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- 5 (i) APPLICANT: CEDARS-SINAI MEDICAL CENTER
- (ii) TITLE OF INVENTION: METHODS OF SCREENING FOR ULCERATIVE COLITIS AND CROHN'S DISEASE
- (iii) NUMBER OF SEQUENCES: 7
- (iv) CORRESPONDENCE ADDRESS:
- 10 (A) ADDRESSEE: PRETTY, SCHROEDER, BRUEGGEMANN & CLARK  
(B) STREET: 444 SOUTH FLOWER STREET, SUITE 2000  
(C) CITY: LOS ANGELES  
(D) STATE: CALIFORNIA  
(E) COUNTRY: USA  
(F) ZIP: 90071
- 15 (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- 20 (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER: US 08/196,003  
(B) FILING DATE: 11-FEB-1994  
(C) CLASSIFICATION:
- 25 (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: WHITEFORD, WENDY A.  
(B) REGISTRATION NUMBER: 36,964  
(C) REFERENCE/DOCKET NUMBER: FP07 33198
- (ix) TELECOMMUNICATION INFORMATION:
- (A) TELEPHONE: (213) 622-7700  
(B) TELEFAX: (213) 489-4210

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## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1176 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

## 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCTGAGGTCT TGCCACCTT GACCGTGGGG AGGGGAGAAC CGTCGGTCAC CCGTTCTTGG 60  
AATGGGATGC GACGGTCCAC CTCCCACCCC GTGGGGCCCC GTTGGAGTGG CACCACGACG 120  
AGGCACCCCT CTTCTCGAC TTGCCCCG GTCGACACCC CCTCGGGCGA CTCCAGTGCT 180  
GGTGCCACGA CCACTCCTCT CTAGTGGTAC CTCGGTTAAA GAGCACGGCG TGA CTTGACC 240  
15 TGGACGCCGG GGTTCCCGAC CTCGACAAAC TCTTGTGGAG CCGGGGGATG GTCGAGGTCT 300  
GGAACACTCC TAACTTCTTC GGTGCTCCCT CTTCCACCCC CACCCCATAG GACGTTACGC 360  
CACTGGACAC CGGTGTCCTA GAAACTCTA CCCACACCGG GGCCGATTCC CCACGTACAC 420  
AAGATCCGCA TACACTGGAT CCGACGACTC ACCGGGACCT TCTCCTAGAG CGTCCTCCCC 480  
CTTACTTTAC GGGGTCTCTT CCCGAAGCCC TGCAGGTAGG GACAGACGAG TGTGGAAGA 540  
20 AGAGAGGGAT CAGGACGGTC GCTGAGGGGG TGTGAACAG TCGGGGGCCC AGGATCTCCA 600  
CCTGTGCGTC CCCTGGCACC AGACAAGGA CCTGCCCAC AAGGGTCAGA GCCTCCGGGT 660  
CCAGGTGGAC CGTGACCCCC TGGTCTCCAA CTTGGGGTGT CAGTGGATAC CGTTGCTGAG 720  
GAAGAGCCGG TTCCGGAGTC AGTCACACTG GCGTCTCCTG CTCCCGTGGG TCGCCGACTG 780  
CACACGTCAT TATGACCCCT TGGTCTCGGT CCTCTGTGAC GTCTGTCACT GGTAGATGTC 840  
25 ATTCTTCCCC GTCCCCGCT CACCCCGAAG AACCCCAACA CTGGACTTGG GCCCCGCCCC 900

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GAGTGACACA CGGATAAGGT CCGAAAGGCC GCGGGTTGCA CTAAGACTGC TTCGGTCTCC 960  
 AGAGTCTTCC CTGGCTCCAC TGTCACCTCA CACTCCGGGT GGGATCTCGG TTCCACTGCG 1020  
 ACTTACCCCA AGGTCGGGTC GGTGACCCGG GCTCCCGGGT CGAGGACGAC TTCCGGTGGG 1080  
 GTCTCCTGTT GCCCCGCTCG AAGAGGACGA GACGTTGGGA CCTCCACCGG CCGGTCGAAT 1140  
 5 ATGTGTTCTT GGTCTGGGCC CTCGAAGCAC AGGACA 1176

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1599 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION: 1..1596

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATG GCT CCC AGC AGC CCC CGG CCC GCG CTG CCC GCA CTC CTG GTC CTG 48  
 Met Ala Pro Ser Ser Pro Arg Pro Ala Leu Pro Ala Leu Leu Val Leu  
 1 5 10 15

20 CTC GGG GCT CTG TTC CCA GGA CCT GGC AAT GCC CAG ACA TCT GTG TCC 96  
 Leu Gly Ala Leu Phe Pro Gly Pro Gly Asn Ala Gln Thr Ser Val Ser  
 20 25 30

CCC TCA AAA GTC ATC CGT CCC CGG GGA GGC TCC GTG CTG GTG ACA TGC 144  
 Pro Ser Lys Val Ile Arg Pro Arg Gly Gly Ser Val Leu Val Thr Cys  
 25 35 40 45

AGC ACC TCC TGT GAC CAG CCC AAG TTG TTG GGC ATA GAG ACC CCG TTG 192  
 Ser Thr Ser Cys Asp Gln Pro Lys Leu Leu Gly Ile Glu Thr Pro Leu  
 50 55 60

CCT AAA AAG GAG TTG CTC CTG CCT GGG AAC AAC CCG AAG GTG TAT GAA 240  
 Pro Lys Lys Glu Leu Leu Leu Pro Gly Asn Asn Pro Lys Val Tyr Glu  
 65 70 75 80

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	CTG AGC AAT GTG CAA GAA GAT AGC CAA CCA ATG TGC TAT TCA AAC TGC	288
	Leu Ser Asn Val Gln Glu Asp Ser Gln Pro Met Cys Tyr Ser Asn Cys	
	85 90 95	
	CCT GAT GGG CAG TCA ACA GCT AAA ACC TTC CTC ACC GTG TAC TGG ACT	336
5	Pro Asp Gly Gln Ser Thr Ala Lys Thr Phe Leu Thr Val Tyr Trp Thr	
	100 105 110	
	CCA GAA CGG GTG GAA CTG GCA CCC CTC CCC TCT TGG CAG CCA GTG GGC	384
	Pro Glu Arg Val Glu Leu Ala Pro Leu Pro Ser Trp Gln Pro Val Gly	
	115 120 125	
10	AAG AAC CTT ACC CTA CGC TGC CAG GTG GAG GGT GGG GCA CCC CGG GCC	432
	Lys Asn Leu Thr Leu Arg Cys Gln Val Glu Gly Gly Ala Pro Arg Ala	
	130 135 140	
	AAC CTC ACC GTG GTG CTG CTC CGT GGG GAG AAG GAG CTG AAA CGG GAG	480
	Asn Leu Thr Val Val Leu Leu Arg Gly Glu Lys Glu Leu Lys Arg Glu	
15	145 150 155 160	
	CCA GCT GTG GGG GAG CCC GCT GAG GTC ACG ACC ACG GTG CTG GTG AGG	528
	Pro Ala Val Gly Glu Pro Ala Glu Val Thr Thr Thr Val Leu Val Arg	
	165 170 175	
	AGA GAT CAC CAT GGA GCC AAT TTC TCG TGC CGC ACT GAA CTG GAC CTG	576
20	Arg Asp His His Gly Ala Asn Phe Ser Cys Arg Thr Glu Leu Asp Leu	
	180 185 190	
	CGG CCC CAA GGG CTG GAG CTG TTT GAG AAC ACC TCG GCC CCC TAC CAG	624
	Arg Pro Gln Gly Leu Glu Leu Phe Glu Asn Thr Ser Ala Pro Tyr Gln	
	195 200 205	
25	CTC CAG ACC TTT GTC CTG CCA GCG ACT CCC CCA CAA CTT GTC AGC CCC	672
	Leu Gln Thr Phe Val Leu Pro Ala Thr Pro Pro Gln Leu Val Ser Pro	
	210 215 220	
	CGG GTC CTA GAG GTG GAC ACG CAG CGG ACC GTG GTC TGT TCC CTG GAC	720
	Arg Val Leu Glu Val Asp Thr Gln Arg Thr Val Val Cys Ser Leu Asp	
30	225 230 235 240	
	GGG CTG TTC CCA GTC TCG GAG GCC CAG GTC CAC CTG GCA CTG GGG GAC	768
	Gly Leu Phe Pro Val Ser Glu Ala Gln Val His Leu Ala Leu Gly Asp	
	245 250 255	

- 44 -

	CAG AGG TTG AAC CCC ACA GTC ACC TAT GGC AAC GAC TCC TTC TCG GCC	816
	Gln Arg Leu Asn Pro Thr Val Thr Tyr Gly Asn Asp Ser Phe Ser Ala	
	260 265 270	
	AAG GCC TCA GTC AGT GTG ACC GCA GAG GAC GAG GGC ACC CAG CGG CTG	864
5	Lys Ala Ser Val Ser Val Thr Ala Glu Asp Glu Gly Thr Gln Arg Leu	
	275 280 285	
	ACG TGT GCA GTA ATA CTG GGG AAC CAG AGC CAG GAG ACA CTG CAG ACA	912
	Thr Cys Ala Val Ile Leu Gly Asn Gln Ser Gln Glu Thr Leu Gln Thr	
	290 295 300	
10	GTG ACC ATC TAC AGC TTT CCG GCG CCC AAC GTG ATT CTG ACG AAG CCA	960
	Val Thr Ile Tyr Ser Phe Pro Ala Pro Asn Val Ile Leu Thr Lys Pro	
	305 310 315 320	
	GAG GTC TCA GAA CGG ACC GAG GTG ACA GTG AAG TGT GAG GCC CAC CCT	1008
15	Glu Val Ser Glu Arg Thr Glu Val Thr Val Lys Cys Glu Ala His Pro	
	325 330 335	
	AGA GCC AAG GTG ACG CTG AAT GGG GTT CCA GCC CAG CCA CTG GGC CCG	1056
	Arg Ala Lys Val Thr Leu Asn Gly Val Pro Ala Gln Pro Leu Gly Pro	
	340 345 350	
	AGG GCC CAG CTC CTG CTG AAG GCC ACC CCA GAG GAC AAC GGG CGC AGC	1104
20	Arg Ala Gln Leu Leu Leu Lys Ala Thr Pro Glu Asp Asn Gly Arg Ser	
	355 360 365	
	TTC TCC TGC TCT GCA ACC CTG GAG GTG GCC GGC CAG CTT ATA CAC AAG	1152
	Phe Ser Cys Ser Ala Thr Leu Glu Val Ala Gly Gln Leu Ile His Lys	
	370 375 380	
25	AAC CAG ACC CGG GAG CTT CGT GTC CTG TAT GGC CCC CGA CTG GAC GAG	1200
	Asn Gln Thr Arg Glu Leu Arg Val Leu Tyr Gly Pro Arg Leu Asp Glu	
	385 390 395 400	
	AGG GAT TGT CCG GGA AAC TGG ACG TGG CCA GAA AAT TCC CAG CAG ACT	1248
30	Arg Asp Cys Pro Gly Asn Trp Thr Trp Pro Glu Asn Ser Gln Gln Thr	
	405 410 415	
	CCA ATG TGC CAG GCT TGG GGG AAC CCA TTG CCC GAG CTC AAG TGT CTA	1296
	Pro Met Cys Gln Ala Trp Gly Asn Pro Leu Pro Glu Leu Lys Cys Leu	
	420 425 430	

- 45 -

	AAG GAT GGC ACT TTC CCA CTG CCC ATC GGG GAA TCA GTG ACT GTC ACT	1344
	Lys Asp Gly Thr Phe Pro Leu Pro Ile Gly Glu Ser Val Thr Val Thr	
	435 440 445	
5	CGA GAT CTT GAG GGC ACC TAC CTC TGT CGG GCC AGG AGC ACT CAA GGG	1392
	Arg Asp Leu Glu Gly Thr Tyr Leu Cys Arg Ala Arg Ser Thr Gln Gly	
	450 455 460	
	GAG GTC ACC CGC GAG GTG ACC GTG AAT GTG CTC TCC CCC CGG TAT GAG	1440
	Glu Val Thr Arg Glu Val Thr Val Asn Val Leu Ser Pro Arg Tyr Glu	
	465 470 475 480	
10	ATT GTC ATC ATC ACT GTG GTA GCA GCC GCA GTC ATA ATG GGC ACT GCA	1488
	Ile Val Ile Ile Thr Val Val Ala Ala Ala Val Ile Met Gly Thr Ala	
	485 490 495	
	GGC CTC AGC ACG TAC CTC TAT AAC CGC CAG CGG AAG ATC AAG AAA TAC	1536
	Gly Leu Ser Thr Tyr Leu Tyr Asn Arg Gln Arg Lys Ile Lys Lys Tyr	
15	500 505 510	
	AGA CTA CAA CAG GCC CAA AAA GGG ACC CCC ATG AAA CCG AAC ACA CAA	1584
	Arg Leu Gln Gln Ala Gln Lys Gly Thr Pro Met Lys Pro Asn Thr Gln	
	515 520 525	
	GCC ACG CCT CCC TGA	1599
20	Ala Thr Pro Pro	
	530	

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 532 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

30	Met Ala Pro Ser Ser Pro Arg Pro Ala Leu Pro Ala Leu Leu Val Leu	
	1 5 10 15	
	Leu Gly Ala Leu Phe Pro Gly Pro Gly Asn Ala Gln Thr Ser Val Ser	
	20 25 30	

- 46 -

	Pro Ser Lys Val Ile Arg Pro Arg Gly Gly Ser Val Leu Val Thr Cys	
	35	40 45
	Ser Thr Ser Cys Asp Gln Pro Lys Leu Leu Gly Ile Glu Thr Pro Leu	
	50	55 60
5	Pro Lys Lys Glu Leu Leu Leu Pro Gly Asn Asn Pro Lys Val Tyr Glu	
	65	70 75 80
	Leu Ser Asn Val Gln Glu Asp Ser Gln Pro Met Cys Tyr Ser Asn Cys	
	85	90 95
10	Pro Asp Gly Gln Ser Thr Ala Lys Thr Phe Leu Thr Val Tyr Trp Thr	
	100	105 110
	Pro Glu Arg Val Glu Leu Ala Pro Leu Pro Ser Trp Gln Pro Val Gly	
	115	120 125
	Lys Asn Leu Thr Leu Arg Cys Gln Val Glu Gly Gly Ala Pro Arg Ala	
	130	135 140
15	Asn Leu Thr Val Val Leu Leu Arg Gly Glu Lys Glu Leu Lys Arg Glu	
	145	150 155 160
	Pro Ala Val Gly Glu Pro Ala Glu Val Thr Thr Thr Val Leu Val Arg	
	165	170 175
20	Arg Asp His His Gly Ala Asn Phe Ser Cys Arg Thr Glu Leu Asp Leu	
	180	185 190
	Arg Pro Gln Gly Leu Glu Leu Phe Glu Asn Thr Ser Ala Pro Tyr Gln	
	195	200 205
	Leu Gln Thr Phe Val Leu Pro Ala Thr Pro Pro Gln Leu Val Ser Pro	
	210	215 220
25	Arg Val Leu Glu Val Asp Thr Gln Arg Thr Val Val Cys Ser Leu Asp	
	225	230 235 240
	Gly Leu Phe Pro Val Ser Glu Ala Gln Val His Leu Ala Leu Gly Asp	
	245	250 255
30	Gln Arg Leu Asn Pro Thr Val Thr Tyr Gly Asn Asp Ser Phe Ser Ala	
	260	265 270



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	Lys	Ala	Ser	Val	Ser	Val	Thr	Ala	Glu	Asp	Glu	Gly	Thr	Gln	Arg	Leu	275	280	285
	Thr	Cys	Ala	Val	Ile	Leu	Gly	Asn	Gln	Ser	Gln	Glu	Thr	Leu	Gln	Thr	290	295	300
5	Val	Thr	Ile	Tyr	Ser	Phe	Pro	Ala	Pro	Asn	Val	Ile	Leu	Thr	Lys	Pro	305	310	315 320
	Glu	Val	Ser	Glu	Arg	Thr	Glu	Val	Thr	Val	Lys	Cys	Glu	Ala	His	Pro	325	330	335
10	Arg	Ala	Lys	Val	Thr	Leu	Asn	Gly	Val	Pro	Ala	Gln	Pro	Leu	Gly	Pro	340	345	350
	Arg	Ala	Gln	Leu	Leu	Leu	Lys	Ala	Thr	Pro	Glu	Asp	Asn	Gly	Arg	Ser	355	360	365
	Phe	Ser	Cys	Ser	Ala	Thr	Leu	Glu	Val	Ala	Gly	Gln	Leu	Ile	His	Lys	370	375	380
15	Asn	Gln	Thr	Arg	Glu	Leu	Arg	Val	Leu	Tyr	Gly	Pro	Arg	Leu	Asp	Glu	385	390	395 400
	Arg	Asp	Cys	Pro	Gly	Asn	Trp	Thr	Trp	Pro	Glu	Asn	Ser	Gln	Gln	Thr	405	410	415
20	Pro	Met	Cys	Gln	Ala	Trp	Gly	Asn	Pro	Leu	Pro	Glu	Leu	Lys	Cys	Leu	420	425	430
	Lys	Asp	Gly	Thr	Phe	Pro	Leu	Pro	Ile	Gly	Glu	Ser	Val	Thr	Val	Thr	435	440	445
	Arg	Asp	Leu	Glu	Gly	Thr	Tyr	Leu	Cys	Arg	Ala	Arg	Ser	Thr	Gln	Gly	450	455	460
25	Glu	Val	Thr	Arg	Glu	Val	Thr	Val	Asn	Val	Leu	Ser	Pro	Arg	Tyr	Glu	465	470	475 480
	Ile	Val	Ile	Ile	Thr	Val	Val	Ala	Ala	Ala	Val	Ile	Met	Gly	Thr	Ala	485	490	495
30	Gly	Leu	Ser	Thr	Tyr	Leu	Tyr	Asn	Arg	Gln	Arg	Lys	Ile	Lys	Lys	Tyr	500	505	510

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Arg Leu Gln Gln Ala Gln Lys Gly Thr Pro Met Lys Pro Asn Thr Gln  
515 520 525

Ala Thr Pro Pro  
530

5 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GATTGAAGAA GCCAGCAG

18

(2) INFORMATION FOR SEQ ID NO:5:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTCGTTGCCA TAGGTGAC

8

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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TCCCTGGACG GGCTGTTCC

9

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

5

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCCCTGGACA GGCTGTTCC

9

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## Claims:

1. A method of screening for inflammatory bowel disease ("IBD"), said method comprising assaying nucleic acid of a subject for the presence or absence of R241 allele of the ICAM-1 gene, wherein the presence of said R241 allele is indicative of IBD.
2. A method in accordance with claim 1, wherein said IBD is ulcerative colitis ("UC").
3. A method in accordance with claim 1, wherein said IBD is Crohn's disease ("CD").
4. A method in accordance with claim 1, wherein said assaying comprises amplification of said nucleic acid, wherein said nucleic acid comprises a sequence that encodes at least nucleotide 778 of ICAM-1 cDNA.
5. A method in accordance with claim 4, wherein said amplification comprises polymerase chain reaction using at least one primer selected from the group consisting of SEQ ID NO 4 and SEQ ID NO 5.
6. A method in accordance with claim 5, wherein said primer is SEQ ID NO 4.
7. A method in accordance with claim 5, wherein said primer is SEQ ID NO 5.
8. A method in accordance with claim 1, wherein said assaying comprises:
  - (a) contacting said nucleic acid with an allele-specific oligonucleotide probe under conditions suitable to cause the probe to hybridize with nucleic acid encoding R241 allele of the ICAM-1 gene, but not with nucleic acid encoding G241 allele of the ICAM-1 gene, and

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(b) detecting the presence or absence of hybridization.

9. A method in accordance with claim 8, wherein said probe is about 7 to about 35 nucleotides in length.

10. A method in accordance with claim 8, wherein said probe comprises a sequence complementary to a region genomic DNA encoding R241 allele wherein said region comprises at least the first nucleotide of codon 241.

11. A method in accordance with claim 8, wherein said probe comprises a sequence complementary to a region of SEQ ID NO 2, wherein said region comprises at least nucleotide 778 of SEQ ID NO 2.

12. A method in accordance with claim 1, wherein said assaying comprises:

5 (a) hybridizing said nucleic acid with a pair of oligonucleotide probes to produce a construct, wherein a first probe of said pair is labeled with a first label and a second probe of said pair is labeled with a second label, such that the first label is distinguishable from said second label, and the probes hybridize adjacent to each other at  
10 a nucleotide encoding amino acid residue 241 of ICAM-1;

(b) reacting said construct with a ligase in a reaction medium; and then

(c) analyzing said reaction medium to detect the presence or absence of a ligation product comprising said first probe and said second probe.

13. A method in accordance with claim 1, further comprising detecting the presence or absence of anti-neutrophil cytoplasmic antibody in a sample of said subject.

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14. A kit for use in screening IBD comprising allele-specific oligonucleotide probe immobilized on solid matrix.

15. A kit in accordance with claim 14, said kit further comprising means for amplifying a subject's nucleic acid encoding ICAM-1, wherein said nucleic acid comprises a sequence that encodes at least nucleotide 778 of ICAM-1 cDNA.

16. An antibody specifically reactive with ICAM-1 encoded by an R241 allele of the ICAM-1 gene.

17. An antibody in accordance with claim 16, wherein said antibody is monoclonal.

18. An antibody in accordance with claim 16, wherein said antibody is polyclonal.

19. An antibody in accordance with claim 16, wherein said antibody is humanized.

20. A method for treating IBD, comprising administering a therapeutically effective dose of an antibody which binds R241 ICAM-1, wherein the R241 ICAM-1 function is modified.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 95/01434

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C12Q1/68 C07K14/705 C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C12Q C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CLINICAL RESEARCH 42 (1). JAN. 1994. 76A, YANG H; VORA D; BEAUDET A; TARGAN S; ROTTER J I 'Association of intercellular adhesion molecule -1 ( ICAM -1) gene with subsets of inflammatory bowel disease (BID) stratified by anti neutrophil cytoplasmic antibodies (ANCA's).' see abstract --- -/--	1-20

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- 'A' document defining the general state of the art which is not considered to be of particular relevance
- 'E' earlier document but published on or after the international filing date
- 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search

23 June 1995

Date of mailing of the international search report

21. 07. 95

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Authorized officer

Molina Galan, E

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 95/01434

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	GASTROENTEROLOGY 106 (4 SUPPL.). 1994. A794., YANG H; VORA D; TARGAN S; TOYODA H; BEAUDET A; ROTTER J I 'Genetic heterogeneity with UC and Crohn 's defined by antineutrophil cytoplasmic antibodies (ANCA's) and intercellular adhesion molecule -1 (ICAM-1) polymorphisms.' see abstract ----	1-20
A	CELL, vol. 52, March 1988 NA US, pages 925-933, STAUNTON ET AL. 'Primary structure of ICAM-1 ...' cited in the application see figures 2,4 ----	1-12
A	JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, vol. 86, no. 2, 1990 pages 202-210, SAXON EZ AL. 'A distinct subset...' cited in the application see the whole document ----	13
A	WO,A,91 16928 (CELLTECH LTD ;BOEHRINGER INGELHEIM PHARMA (US)) 14 November 1991 ----	
A	LAB. INVEST., (1993) 69/1 (77-85)., NAKAMURA S. ET AL. 'In situ expression of the cell adhesion molecules in inflammatory bowel disease : Evidence of immunologic activation of vascular endothelial cells. ' ----	
A	CELL, vol. 65, no. 6, 14 June 1991 pages 961-971, XP 000206759 DIAMOND M S ET AL 'BINDING OF THE INTEGRIN MAC-1 (CD11B/CD18) TO THE THIRD IMMUNOGLOBULIN-LIKE DOMAIN OF ICAM-1 (CD54) AND ITS REGULATION BY GLYCOSYLATION' cited in the application -----	



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**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim 20 is directed to a method of treatment of the human /animal body the search has been carried out and based on the alleged effects of the compound/composititon.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

A novel association between inflammatory bowel disease (IBD) and a polymorphism at amino acid residue 241 *off* intercellular adhesion molecule-1 (ICAM-1) has been discovered. In accordance with the present invention there is provided methods of screening for IBD, methods for treating IBD, antibodies specifically reactive with ICAM-1 encoded by R241 allele and kits which exploit the inventive methods.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No

PCT/US 95/01434

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9116928	14-11-91	AU-B- 649682	02-06-94
		AU-A- 7860391	27-11-91
		EP-A- 0528931	03-03-93
		HU-A- 66103	28-09-94
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